Enhancement of reducing sugar production by A. *niger* USM AI1 on oil palm frond in tray system via solid state fermentation

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Abstract. To obtain higher reducing sugar production via solid state fermentation (SSF), it is necessary to develop a stabile bioprocess system. In this study, optimized the physical parameter of SSF in a shallow aluminium tray (30 cm x 20 cm x 6 cm) was carried out in order to maximize reducing sugar production by *A. niger* USM AI1 using oil palm frond as a substrate. Production of reducing sugars before optimization in tray system was 185.00 ± 2.85 mg / g substrate with the fungal growth of 2.32 mg ± 0.06 glucosamine / g substrate. However, after optimization of physical parameter (amount of substrate, inoculum size, temperature and moisture content in substrate) the maximum reducing sugar yield was achieved 251.13 ± 1.95 mg / g substrate with the fungus growth of 3.21 ± 0.10 mg glucosamine / g on the 4 days of cultivation period. The results showed that the production of reducing sugar was increased by 35.7% compared to before optimization. Thus, optimized the physical parameter of solid state fermentation in a tray system has succeeded in increasing the production of reducing sugars. To meet the increasing demands of reducing sugar from biomass, it is necessary to obtain the optimum condition in SSF process.
Keywords: reducing sugar, solid state fermentation, *A. niger* USM AI1, physical parameter

Introduction

Malaysia is an agricultural-based country and produces large quantities of agrowastes, of which the majority is disposed of due to low nutritional qualities. Some of these wastes include oil palm frond (OPF) that usually are disposed or burned in plantation. The major component of OPF is cellulose, followed by hemicelluloses and lignin, besides minor components such as protein, oil and ash that make up the remaining fraction of lignocellulosis biomass (Sjostrom,1981). Therefore, degradation of OPF to monomeric sugars through the concerted action of cellulolytic enzymes has great importance, since sugars can serve as raw material in a number of biotechnological production processes (Juhasz *et al*., 2005).

SSF is defined as the cultivation of microorganisms on solid substrates that deficient in free flowing water. SSF is considered more economical compared to submerged fermentation, mainly due to the substrates (agricultural wastes) are cheap and abundantly found in Malaysia (Pang & Ibrahim, 2005; Gassara *et al*., 2010). Over the past decades, enzymes-based technologies have aroused worldwide research activity. Study on optimization of physical parameter for the production of fermentable sugars on substrates has been particular interest due to the production of enzymes on-site are more economical to degrade the lignocellulosis biomass to monosaccharide compared to commercial enzyme. This study described the investigations of fermentable sugars production by *Aspergillus niger* USM AI1 using OPF as a potential substrate in a tray system. Some of the governing parameters on the production of fermentable sugars in the SSF system were studied.

Materials and Methods

Microorganisms and culture conditions

The fungus used, *A. niger* USMAI1 was maintained on potato dextrose agar slants at 37°C until sporulation for 5 days. The culture was stored at 4°C. The inoculums were prepared by adding sterile distilled water into an agar slant.

Substrate preparation and Solid-state fermentation (SSF)

Oil palm frond was thoroughly dried and milled to desire size. It was then weighed and put into aluminum tray and autoclaved at 121 °C for 20 minutes.

SSF was carried out in different physical parameters (different amount of substrate, inoculum size, initial substrate moisture, incubation temperature).

Fermentable sugars extraction

The crude fermentable sugar from the fermented OPF was extracted by simple contact method. The fermented OPF was mixed thoroughly with distilled water. At the end of extraction, a filter paper was used to filter the suspension and the supernatant was collected and used as the crude fermentable sugar for analysis.

Analyses

The Nelson and Somogyi (Breuil and Saddler, 1984) procedure was used to measure fermentable sugar. One ml crude filtrate solution was added to one ml cuprum reagent. The solution were placed in boiling water for 15 minutes, then cooled it and added 1 ml of Nelson reagent. The solution then added with 10 ml of distilled water and the absorbance of the solution was measured at 540 nm. The fungal biomass was carried out by determining the N-acetyl glucosamine released by the acid hydrolysis of the chitin, present in the cell wall of the fungus (Sakurai *et al*., 1977). Glucosamine released from the chitin by the acid hydrolysis was mixed with 1 ml acetyl acetone reagent and incubated in a boiling water bath for 20 minutes. After cooling, 6 ml ethanol was added followed by the addition of 1 ml of Ehrlich reagent and incubated at 65°C for 10 minutes. After cooling the optical density at 530 nm was taken against the reagent blank.

Results and Discussion

The results revealed that, after optimization of the physical parameters (Table 1) the maximal activity of fermentable sugars of 251.13 ± 1.95 mg/g substrate was obtained. Figure 1 and 2 shows the profile of fermentable sugars and fungal growth production before (Figure 1) and after (Figure 2) optimization of physical parameters. There were about 35.7 % increases of fermentable sugar production after optimization (251.13 ± 1.95 mg/g substrate) compared to before optimization (185.00 ± 2.85 mg/g substrate). The results indicated that the physical parameters could influence the productivity of fermentable sugars significantly. Physical factors like temperature, moisture, and inoculum size can play an important role for the adherence of fungus to solid substrate, which in turn affects to the production of enzyme and it may affect production of fermentable sugars (Mulimani *et al*., 2000).

Table 1. The optimum of physical parameter conditions for the production of reducing sugars from OPF by *A.niger* USMAI1 in tray system

Parameter	**Optimum condition**
1. Amount of substrate (OPF)	60(g)
2. Inoculum size	$1x10^7$ spore/ml
3. Moisture	180 % (v/w)
4. Temperature	Room temperature (30±2ºC)

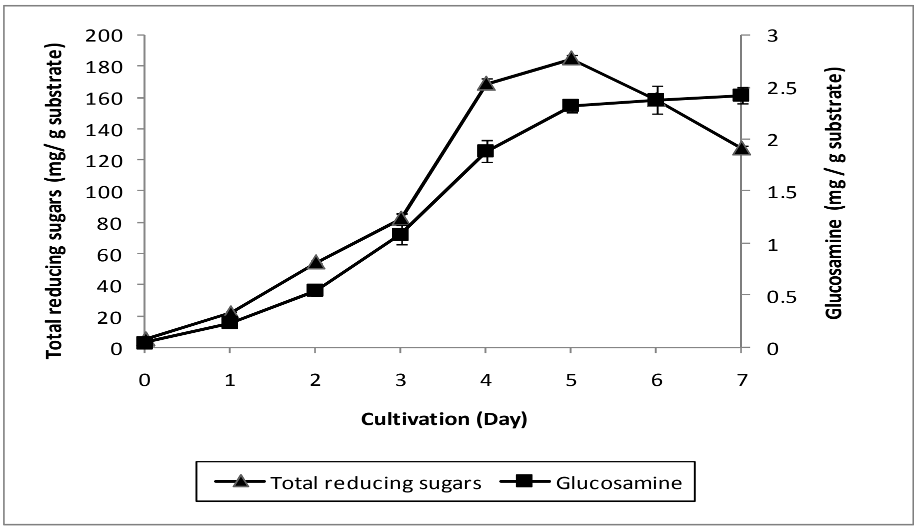

Figure 1. Profile of reducing sugars production and fungal growth of *A. niger* USM AI1 before optimization of physical parameter in tray system

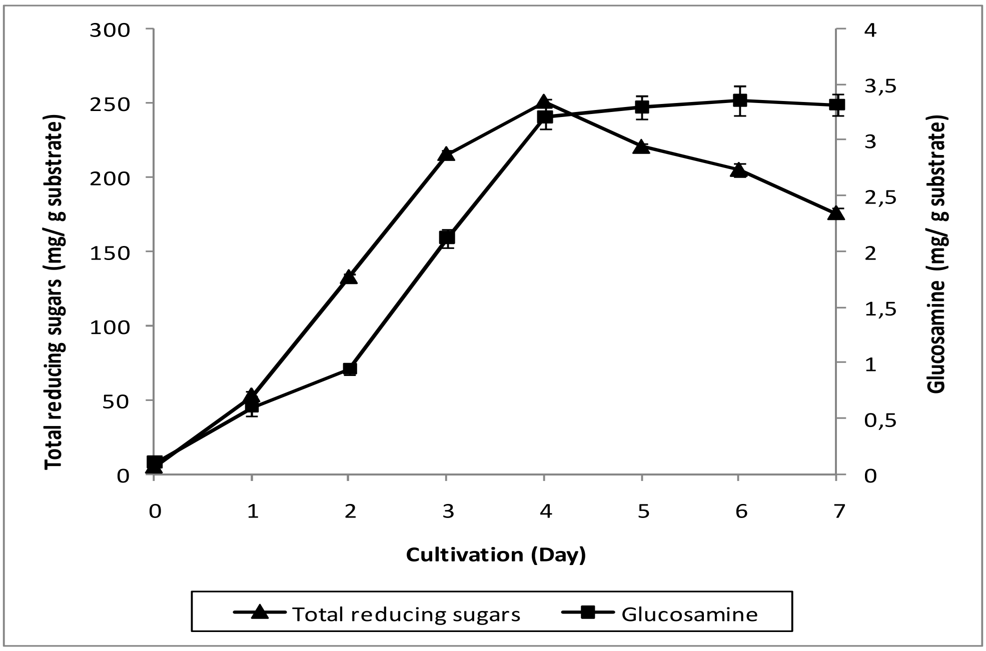

Figure 2. Profile of reducing sugars production and fungal growth of *A. niger* USM AI1 after optimization of physical parameter in tray system

Conclusions

It is noted from this study the results demonstrate the importance of physical parameters in solid state fermentation culture in obtaining good product yield of fermentable sugars in tray system.

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References

Breuil C., Saddler N. 1984. Comparision of the 3,5-dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity. Enzyme and Microbe. Technology 7: 327-332.

Gassara F., Brar S.K., Tyagi R.D., Verma M., Surampalli R.Y. 2010. Screening of agroindustrial waste to produce ligninolytic enzymes by Phanerochaete chrysosporium. Biochemical Engineering Journal 49: 388-394.

Juhasz T., Szengyel Z., Reczey K., Silika-Aho M., Viikari. 2005. Characterization of cellulase and hemicellulases produced by Trichoderma reesei on various carbon source. Process Biochemistry(Article in press) 1-7.

Mulimani V.H., Patil G.N., Ramalingam. 2000. α-Amylase production by solid-state fermentation: a new practical approach to biotechnology course. Biochemical Education 28: 161-163.

Pang P.K., Ibrahim, C.O. 2005. Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. Songklanakarin Journal of Science Technology 27: 325-336.

Sakurai Y., Lee T.H., Shiota H. 1977. On the convenient method of the glucosamine estimation in koji. Agricutural and Biological Chemistry 41: 619-624.

Sjostrom E. 1981. Wood chemisty. In: *Fundamentals And Applications*. New York: Academic Press Inc., p. 223.